Comparison of Cd Binding Mechanisms by Gram-Positive, Gram-Negative and Consortia of Bacteria Using XAFS

Bhoopesh Mishra[1], Jeremy B. Fein[2], Maxim I. Boyanov[3], Shelly D. Kelly[3], Kenneth M. Kemner[3] and Bruce A. Bunker[1]

[1]*Department of Physics, University of Notre Dame, Notre Dame, IN 46556 USA*
[2]*Department of Civil Engineering and Geological Sciences, University of Notre Dame, Notre Dame, IN 46556 USA*
[3]*Biological Sciences, Argonne National Laboratory, Argonne, IL 60439 USA*

Abstract. A quantitative comparison of the Cd binding mechanism to Gram-positive (*Bacillus subtilis)* and Gram-negative bacteria (*Shewanella oneidensis)* is presented. At pH 6.0, EXAFS data for the Gram-positive bacteria were modeled using carboxyl and phosphoryl sites only. However, additional sulfide sites were required to model the spectrum from the Gram-negative bacteria under similar experimental conditions. Cd binding to a bacterial consortium at the same pH value, sampled from natural river water, was modeled using the models developed for the individual Gram-positive and Gram-negative bacterial strains.

Keywords: XAFS, Cd, bacteria**,** Gram positive, Gram negative, consortium, adsorption X-ray absorption spectroscopy: EXAFS, NEXAFS, XANES
PACS: 61.10.Ht

INTRODUCTION

Adsorption onto bacterial surfaces can control the speciation and distribution of contaminants in many aquatic and near-surface systems. Accurate models that describe bacteria-metal interactions are critical to understanding the behavior of heavy metal contaminants and the development of contaminant remediation strategies. An obstacle in modeling realistic systems is that a given bacteria-bearing natural system can contain many different bacterial species. However, recent studies have shown that individual pure strains of Gram-positive and Gram-negative bacteria and their artificial mixtures exhibit broadly similar adsorptive behavior [1, 2, 3]. Similarly, Borrok *et al.* found that consortia of bacteria grown from a range of uncontaminated soil and water environments exhibit roughly similar affinities for protons and Cd [4]. In this study, XAFS has been used to compare the Cd binding mechanism of a Gram-positive bacterium (*Bacillus subtilis*) with a Gram-negative bacterium (*Shewanella oneidensis*) at pH 6.0. Further, the Cd binding mechanism of an uncontaminated river water bacterial consortium has been compared with the two pure bacterial strains under similar experimental condition. DGGE analysis of the river water consortium sample shows the presence of at least six different bacterial species [4]. Our study will help to resolve whether binding sites determined for single species systems are responsible for adsorption in more complex natural bacterial assemblages.

METHODS

The river water sample that was used in this study was collected from the St. Joseph River in South Bend, IN, USA. *Bacillus subtilis*, *Shewanella oneidensis* and the bacterial consortium were harvested from the TSB growth media by centrifugation, transferred to test tubes, and washed five times in 0.1M $NaClO_4$. In each Cd adsorption experiment, 10g/L of bacterial wet weight was suspended in a pH-neutralized stock solution of 0.1 mol/L $NaClO_4$ and 30 ppm Cd. After adjustment of the pH, and an additional 2 h of reaction time on a rotating rack, the final pH of each vessel was measured. The individual vessels were then centrifuged. The filtered supernatant was analyzed for Cd using an inductively coupled plasma-atomic emission spectroscopy technique with matrix-matched standards. The biomass pellet formed at the base of each vessel after centrifugation was loaded into slotted Plexiglas holders and covered with Kapton film for XAFS measurements.

CP882, *X-ray Absorption Fine Structure—XAFS13*
edited by B. Hedman and P. Pianetta

Powder and aqueous Cd standards were used to determine the XAFS signature of carboxyl, phosphoryl, sulfide and sulfate binding environments. CdS and $CdSO_4$ powder standards were prepared from commercially available chemicals (Sigma-Aldrich), after grinding and sieving (~ 400 mesh). The aqueous Cd standards include hydrated Cd, Cd acetate and Cd phosphate solutions. All Cd standards were prepared from 1000 ppm Cd perchlorate stock solution. pH of the solution standards were adjusted such that complexation of Cd to the ligands was expected from solution speciation calculations.

XAFS measurements of Cd K edge (26711 eV) were performed at the MRCAT sector 10-ID beamline [5] at the Advanced Photon Source at Argonne National Laboratory. The energy of the incident X-rays was scanned by using a Si(111) reflection plane of a cryogenically-cooled double-crystal monochromator. The beamline was optimized at the 3rd harmonic of the undulator. The undulator was tapered by approximately 3.5 keV to reduce the variation in the incident intensity to less than 15% over the scanned energy range. Higher harmonics were rejected using a Pt-coated mirror. The incident ion chamber was filled with 100% Nitrogen. The transmitted and reference ion chambers were filled with 100% Ar. The fluorescence detector in the Stern-Heald geometry [6] was filled with Kr gas, and Pd filter of three absorption lengths was used to reduce the background signal. The incident X-ray beam profile was 1 mm square. Linearity tests [7] indicated less than 0.1% nonlinearity for a 50% decrease in incident X-ray intensity. The scans were aligned by the simultaneously collected Cd foil data. The first inflection point was set at 26711 eV.

Quick scans (continuous scanning of the monochromator with signal sampled every 0.5 eV in the entire scanning range) were used with an integration time of 0.1 second per point. About 50 consecutive scans of each sample were averaged, and resulting data from all the samples were normalized and background subtracted using ATHENA [8].

The data were analyzed using codes from the UWXAFS package [9]. Data range used for Fourier transforming the k space data was 2.3 – 9.8 $Å^{-1}$. A Hanning window function was used with a δk of 1.0 $Å^{-1}$. The Gram-positive, Gram-negative, and river water consortium samples were first fit individually at k weights 1, 2, and 3, and then a simultaneous fitting of these three samples was done. Only four SS paths, Cd-O, Cd-C, Cd-P, and Cd-S were used to fit the biomass samples. These paths were first used to fit hydrated Cd, Cd acetate, Cd phosphate and Cd sulfide to calibrate the theory (not shown). A shell-by-shell fitting approach was used, in which significantly smaller χ_v^2 and R factor values were used as the criteria for the goodness of fit. The fitting range for all the data sets were set to 1.2 – 3.4 Å. In the simultaneous fit of all three samples, the Debye-Waller factors were set to the optimized values in the fits of the individual samples.

RESULTS AND DISCUSSION

The experimental $k^2\chi$ data for the three samples are shown in Fig. 1. Corresponding magnitude of the Fourier transform and the fits are shown in Fig. 2. The differences between these three spectra are more clearly seen in the real part of the FT, shown in Fig. 3.

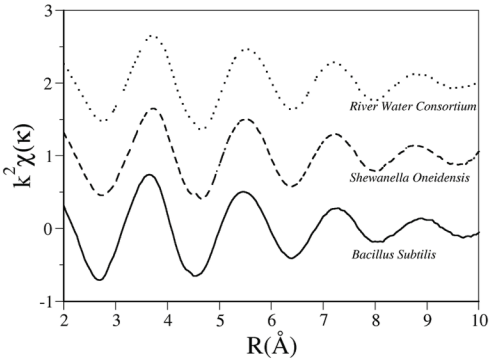

FIGURE 1. $k^2\chi$ data of the Gram positive, Gram negative and river water consortium of bacteria at pH 6.0.

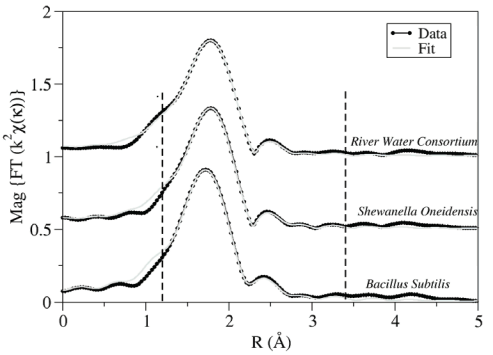

FIGURE 2. Data and Fit for the magnitude of the Fourier transform of the the Gram positive, Gram negative and bacterial consortium at pH 6.0.

The Gram positive bacterial sample (*B. subtilis*) was fit using carboxyl and phosphoryl sites, consistent with previous results [10]. An attempt was made to refine a sulfide site to that data, but the fit produced a coordination number of only 0.08±0.05 sulfur atoms. Conversely, the Gram negative bacterial sample *Shewanella oneidensis* could not be modeled using carboxyl and phosphoryl sites alone. Adding a sulfide site to the model significantly improved the fit, reducing χ_v^2 from 110 to 35. Data from the river water consortium sample was successfully fit using the same

paths used for the two pure bacterial strains, producing the parameters in Table 1 and a χ_v^2 value of 30.

Figure 4 makes it clear that the Cd-S path has significant contribution in the EXAFS signal of the Gram-negative bacteria and the river water consortium. The fitting details are shown in Table 1.

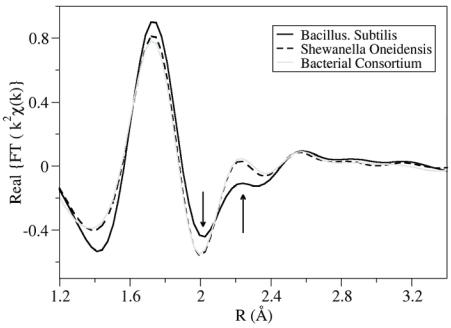

FIGURE 3. Comparing the real part of the FT of *Bacillus subtilis*, *Shewanella oneidensis* and the river water consortium. It can be clearly seen that *B. subtilis* data is significantly different around 2.2 Å, while *Shewanella oneidensis* and the river water consortium are similar.

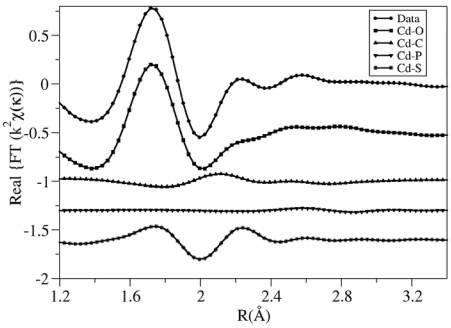

FIGURE 4. Real part of the FT data of the river water consortium and contribution of the four paths (Cd-O, Cd-C, Cd-P, and Cd-S) in fitting this data. Notice the relatively strong sulfide contribution.

TABLE 1. XAFS Fitting Parameters

Sample/Path	N	R(Å)	σ^2 (Å^{-2})×10^{-3}
Cd/*B. subtilis*			
Cd-O	4.78 ± 0.12	2.29 ± 0.01	9.00
Cd-C	0.89 ± 0.46	2.70 ± 0.03	12.00
Cd-P	0.82 ± 0.32	3.38 ± 0.05	15.00
Cd-S	0.08 ± 0.05	2.53 ± 0.02	9.00
Cd/*Shewanella*	N	R(Å)	σ^2 (Å^{-2})×10^{-3}
Cd-O	3.72 ± 0.22	2.29 ± 0.01	9.00
Cd-C	1.30 ± 0.56	2.70 ± 0.03	12.00
Cd-P	0.58 ± 0.30	3.38 ± 0.05	15.00
Cd-S	0.90 ± 0.16	2.53 ± 0.02	9.00
Cd/*Consortium*	N	R(Å)	σ^2 (Å^{-2})×10^{-3}
Cd-O	3.33 ± 0.25	2.29 ± 0.01	9.00
Cd-C	0.91 ± 0.49	2.70 ± 0.03	12.00
Cd-P	0.65 ± 0.30	3.36 ± 0.05	15.00
Cd-S	1.14 ± 0.10	2.53 ± 0.02	9.00

In summary, this study demonstrates that Cd adsorption to Gram-positive bacteria is different than Gram-negative bacteria under similar experimental conditions. While Gram-positive bacteria could be modeled using carboxyl and phosphoryl sites only, an additional, sulfide site was required for modeling the Gram-negative bacteria. We also demonstrate that a natural consortium of bacteria sampled from uncontaminated river water, containing at least six different bacterial species, can be modeled using the models developed for the individual Gram-positive and Gram-negative bacterial strains. The XAFS data from the bacterial consortium were similar to that of the Gram-negative bacteria. The possibility of this consortium being dominated Gram-negative bacteria cannot be ruled out. Nevertheless, it can be inferred from our results that Cd adsorption to bacterial consortia could be modeled using models developed for individual bacterial strains. However, this study needs to be extended to a range of pH values and Cd loadings on several Gram-positive, Gram–negative, and bacterial consortium for more reliable interpretation of our results.

ACKNOWLEDGMENTS

B.M. thanks the Bayer Corporation and the Environmental Molecular Science Institute (EMSI) at University of Notre Dame for fellowship support. Help in sample preparation from Jennifer Szymanowski, and beamline setup help from the staff of MRCAT are greatly appreciated. This work was supported by the funding provided by the National Science Foundation through an EMSI grant (EAR02-21966) to the University of Notre Dame. MRCAT is supported by the member institutions. The APS was supported by the U.S. Department of Energy, Office of Science and Office of Basic Energy Sciences.

REFERENCES

1. C. J. Daughney, *et al.*, *Chemical Geology* **144,** 161 (1998).
2. N. Yee and J. Fein, *Geochimica et Cosmochimica Acta* **65,** 2037 (2001).
3. N. Yee and J. B. Fein, *Geomicrobiology Journal* **20,** 43 (2003).
4. D. Borrok, *et al.*, *Geochimica et Cosmochimica Acta* **68,** 3231 (2004).
5. C. U. Segre *et al.*, 521 (American Institute of Physics, New York, 2000), p. 419-422.
6. E. A. Stern and S. M Heald, *Handbook of Synchrotron Radiation*, North-Holland, Amsterdam, 1983).
7. K. M. Kemner *et al.*, *Phys. Rev. B* **50,** 14327 (1994).
8. B. Ravel, *Journal of Synchrotron Radiation* **8,** 314 (2001).
9. E. A. Stern *et al.*, *Physica B* **209,** 117 (1995).
10. M. I. Boyanov et al, *Geochimica et Cosmochimica Acta* **67,** 3299 (2003).